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ORIGINAL PAPER

Variability in permeability and integrity of cell membrane and depletion of food reserves in neem (*Azadirachta indica*) seeds from trees of different age classes

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Abstract: We quantified cell membrane permeability (electrical conductivity-EC, water soluble sugar-WSS, and amino acids-AA) and integrity (phospholipids, α-tocopherol and lipid peroxidation) along with food reserve deterioration (total proteins, total sugar, and total starch) of neem seeds collected from various mother tree age classes and stored for 65 days in airtight plastic containers at ambient room temperature (35±5°C). Results show that the activities were higher in fresh seeds (EC 267.56–2950.01 μS/g, WSS 19.96–19.48 mg/g and AA 5.40–5.35 mg/g) and declined with increasing duration of storage period (EC 153.37–195.17 μS/g, WSS 3.13–4.17 mg/g and AA 4.29–4.49 mg/g after 35 days and EC 144.02–161.56 μS/g, WSS 2.06–2.40 mg/g and AA 3.98–4.27 mg/g after 65 days of storage). Phospholipids and α-tocopherol were higher in fresh seed (0.073–0.093 OD at 710 nm and 0.080–0.105 OD, respectively) and declined as storage duration increased (0.033–0.042 OD at 710 nm and 00.0010–0.0020 OD, respectively). Dead seeds showed reduced amounts of phospholipids and minimum activity of α- tocopherol (antioxidants). The level of MDA was lower in fresh seeds (0.0066–0.0087 OD at 600–535 nm) and increased as storage duration increased (0.0248–0.0268 OD after 65 days of storage). The higher amount of MDA indicated that seeds died due to rancidity of the oil inside the seed. Neem seed cake was assessed for deterioration of food reserves (total proteins, total sugar, and total starch), concentrations of which were higher in fresh seed and declined as storage duration increased. Germination was higher in fresh seeds and after 65 days, no germination was received perhaps due to deterioration of biochemicals in seeds. Patterns of seed deterioration were similar across all seed lots.

Keywords: phospholipids, α-Tocopherol, Lipid peroxidation, *Azadirachta indica*

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Introduction

Neem (*Azadirachta indica* A. Juss.) is a multipurpose tree for centuries in the Indian subcontinent. Neem seeds lose their viability rapidly (Ezumah 1986; Nagavani et al. 1987) due to changes in biochemical composition (Pukacka and Ratajczak 2007). The most important change during seed deterioration is alteration of the cell membrane, which affects seed vigor. The plasma lemma designates the cell inner membrane, while the term cell membrane is more generally used for the entire membrane system of the cell. The decline in seed vigor is associated with weakening of the cell membrane (Heydecker 1972). The electrical conductivity of solutions in which plant tissues are bathed, increases with tissue age. Abdul Baki and Anderson (1972) postulated that the decline in seed vigor is associated with integrity of the cell membrane. The observation that leakage during imbibition was similar for high and low vigor wheat (Mukhtar and Laidman 1982) stresses the need for caution regarding conclusions drawn from conductivity tests. In this regard, there is no evidence suggesting that different seed types or organs within individual seed, deteriorate uniformly. Kaloyereas (1958) was the first to suggest that lipid oxidation may underlie loss of seed viability. Koostra and Harrington (1969) analysed the phospholipid changes and raised the possibility that membrane peroxidative changes were associated with ageing. Since that time many studies have sought evidence for changes in membrane phospholipids, but results have, to date, been inconclusive (Bewley 1986; Priestley 1986; Wilson and McDonald 1986). While evidence for lipid oxidation as a primary cause of seed ageing may not be convincing, it does seem clear that high seed moisture content and elevated temperatures lead to marked changes in membrane phospholipids. In most cases the concen-

trations of phospholipids in seeds has been shown to decline with seed age.

Many biochemical changes occur in deteriorating seeds, but it is difficult to distinguish primary from secondary events. Studies of seed deterioration have used many techniques to examine stages of degeneration for a wide variety of seeds (Bewley and Black 1982). The cause of varying vigor and consequent loss of viability in seeds has been related to weakening of cell membranes, mechanical damage or low metabolic activity. Increasing quantities of cellular constituents can be leached from seeds as they deteriorate due to ageing or sudden trauma. Quantitative measurement of the leachates, e.g. electrical conductivity (EC), can be related to seed quality and used to predict germination percentage in a rapid, non-destructive test. The leaching of organic and inorganic constituents during imbibitions is affected by deterioration (Helmer et al. 1962; Ching and Schoolcraft 1968; Bradnock and Matthews 1970; Pehap 1973). Rates of leaching of sugars (Takayanagi and Murakami 1968) and electrolytes (Matthews and Brandnock 1967) have been quantified as rapid tests for seed viability. The leaching rates and the nature of leachates vary depending on the type of seed and type of damage sustained by the seed.

Seeds contain some endogenous antioxidants, which interrupt chain reactions by combining with free radical intermediates (Tappel 1965; Barber and Bernheim 1967) and becoming oxidized. Tocopherols, especially α-tocopherol, form the major antioxidant group associated with both membrane and storage of lipids in seed (Yamauchi and Matsushita 1976; Fielding and Goldsworthy 1980). Several attempts have been made to correlate tocopherol level with seed aging.

One simple method for determining viability is to subject seeds to electrical current and then record the responses of living and dead seeds. Fick and Hibbard (1952) suggested the more reliable electrical method of soaking a seed sample in water for few hours under controlled temperature conditions. The conductivity of the resulting solution reflected the general level of viability of the seed sample. An association between the readiness with which solutes leach from different lots of vining pea seeds and their field germination was first reported by Matthews and Whitbread (1967). This was later developed as a routine test for the prediction of field emergence (Matthews and Bradnock 1967) in which electrolytes leached into water were measured with an electrical conductivity meter.

The seeds of neem typically lose viability within a few days. We investigated the biochemical seed deterioration of different mother tree age classes of neem seeds and its impact on germination during storage. Our objectives were to understand and quantify the cell membrane, cell integrity and food reserves of neem seeds during storage.

Material and methods

Neem seeds were collected from various mother tree age classes i.e. Age I (6 years), Age II (15 years), Age III (25 years), and Age IV (>30 years) near Jodhpur, Rajasthan, India (latitude 24°40′ N and longitude 71°15′ E) during 2002. Seed testing was carried out in the nursery at Arid Forest Research Institute, Jodhpur, Rajasthan, India. Mean minimum and maximum temperatures of 22±6°C and 34±8°C and humidity of 23±10%–54±12% were recorded during the study period.

Freshly collected fruits were kept in gunny bags at ambient room temperature (35±5°C) for one day to soften the pulp. Fruits became soft within one day. Seeds were depulped completely from the endocarp by macerating into cotton bags (Size 30 × 30 cm). Cleaned seeds obtained from all mother tree age classes were shade dried in the laboratory for seven days to reach EMC (equilibrium moisture content) of 7%. Dried seeds were then subjected to storage treatments in 200 μ thick polythene bags and kept in airtight plastic containers at ambient room temperature (35±5°C) for 65 days. Seeds were sown in the nursery when fresh (storage period of 0 days), and after 35 and 65 days of storage for evaluation of viability in nursery beds (size 5.0 m ×1.0 m × 0.45 m) filled with pure sand. Germination data were recorded for 21 days after sowing of seed. A seed was considered germinated when the hypocotyl emerged 1 cm above the sand surface. Germination percentage (ISTA 1993) and rate of germination in terms of mean germination time (MGT) (Rawat and Thapliyal 2003) were calculated while germination value (GV) was calculated following Djavanshir and Pourbeik (1976).

Extraction of oil

Four replications of 300 g of dried seeds were taken for oil extraction from each age class as fresh, and after 35 and 65 days of storage. Seeds were oven dried at 80±1°C for 72 h to remove moisture and then dehusked to yield kernels. The kernels were ground using pestle and mortar. Seed kernel powder was taken for extraction of oil (AOAC 1990) in 500-ml extractor of soxhlet apparatus with petroleum ether (60°C–80°C). Soxhlet was run for 24 h. Extract was then dried over anhydrous sodium sulphate. The solvent was removed by distillation in rotary evaporator to separate fatty oil and total fatty oil yield was calculated. After extraction of oil from seeds of all mother tree age classes, a cell membrane integrity test was performed to quantify the deterioration of seed during storage.

Cell membrane permeability test during storage

The cell membrane permeability was assessed by analyzing the seed leachates, which were various types of solutes, including electrolytes, water-soluble sugars and amino acids.

(1) Electrical Conductivity (EC): Electrical conductivity of seed leachate was measured as per the method of Agrawal and Dadlani (1992).

(2) Water-Soluble Sugars (WSS): One ml of seed leachate was analyzed by using the method described by Dubois et al. (1956). Absorbance was recorded at 490 nm using a UV-VIS Spectrophotometer. The standard curve was prepared using 10 to 100 μg of glucose per ml.

(3) Amino Acids: Amino acids in seed leachates were quantified using the method described by Agrawal and Dadlani (1992). Absorbance was recorded at 570 nm in UV-VIS Spectropho-

tometer. Glycine was used as the standard.

Cell membrane integrity tests during storage

The term "plasma lemma" designates the cell inner membrane, while the term "cell membrane" is more generally used for the entire membrane system of the cell. The following tests were performed to quantify the integrity of the plasma lemma in various seed lots.

(1) Estimation of Phospholipids: The amount of phospholipid in oil was determined following Raheja et al. (1973). The optical density was read at 710 nm in UV-VIS Spectrophotometer.

(2) Estimation of Lipid Peroxidation: Lipid peroxidation of unsaturated fatty acids in the phospholipid fraction of the cell membrane is considered to be one of the major changes associated with seed deterioration. Rancidity was measured to estimate peroxidation level. When unsaturated fatty acids with double bonds three carbon atoms apart (linolenic acid) are subjected to peroxidation, a product is malondiadehyde (MDA) (Steward and Bewley 1980). If peroxidation of unsaturated fatty acids was one of the prime causes of seed deterioration, the level of MDA would be expected to rise with seed age. The level of MDA was determined following Dadlani and Agrawal (1983).

(3) Estimation of Antioxidants (α-Tocopherol): Since peroxidation-degraded polyunsaturated fatty acids are closely associated with seed ageing, exogenous application of antioxidant was shown to improve seed through the maintenance of longevity (Dadlani and Agrawal 1983). This suggests a protective role played by these chemicals in the process of ageing. Presumably to check peroxidation, seeds contain some endogenous antioxidants that interrupt chain reactions by combining with free radical intermediates and becoming oxidized. Tecopherols (especially α-tocopherol or vitamin E) form the major antioxidant group associated with both membrane and storage lipids in seeds. The estimation of antioxidants (α-tocopherol) was performed in three steps viz. oil extraction, saphonification of oil and estimation of tocopherol in the unsaphonified fraction.

Step I – Oil Extraction: Followed the soxhlet method as described earlier.

Step II- Saphoniphication: The saphonification of oil was done following Walker and Singer (1975).

Step III- Quantification of Tocopherols: One ml of the above-mentioned solution was analyzed following Pearson (1970). Absorbance was recorded at 520 nm. α-tocopherol was used as the standard.

Quantification of food reserves during storage

(1) Estimation of Protein: The protein content in deoiled cake was estimated following the method of Lowry et al. (1951). Absorbance was recorded at 750 nm. Albumin was used as the standard.

(2) Estimation of total Sugar: The total sugar content of deoiled cake was estimated following the method of Dubois et al. (1956). Absorbance was recorded at 490 nm. Glucose was used as the standard.

(3) Estimation of Starch: Starch content was estimated in deoiled neem cake following Colowick and Kaplan (1957) and Dubois et al. (1956). Absorbance was recorded at 490 nm in a UV-VIS Spectrophotometer. The standard curve was prepared using 10 to 100 μg of glucose per ml. The calculation of starch was:

$$\text{Starch} = \text{Glucose} \times 0.90$$

Data were analyzed using ANOVA in the SPSS computer package. Statistical significance was set at $\alpha = 0.05$.

Results and discussion

Electrical conductivity of seed leachate did not vary by mother tree age class. From its peak levels in fresh seeds, EC declined with increasing duration of storage (Table 1). This confirmed the result of Yaklich and Abdul-Baki (1975) in soybean, Agrawal (1977) in rice, Bradnock and Matthews (1970) and Perry (1987) in pea and Hsu et al. (2000) in Sudan grass. These authors also reported a negative correlation between germination and EC. No such correlation was reported in musk melon (Pesis and Ng 1983) or in barley (Abdul-Baki and Anderson 1970). Loss of membrane integrity as a consequence of decline in vigor could also be attributed to perturbation to the normal membrane in severely deteriorated seeds such as those subjected to accelerated aging. It is expected that repair of damaged membrane, i.e. the synthetic mode of turnover, will be severely impaired as an indirect consequence of loss of metabolic integrity. Membrane repair can occur when seeds are hydrated slowly. Unaged seeds, which are hydrated in atmospheres of high relative humidity before being placed in water, do not leak appreciable quantities of solute (Simon and Harun 1972). Slow initial hydration of seeds probably allows for ordered rearrangement of the membrane before the inrush of water associated with imbibition from the dry state, which might otherwise be too sudden and disruptive (Powell and Matthews 1977). Storage of seeds in an imbibed state in which they remained metabolically active (Powell et al. 1983 and 1984) can help to maintain seed viability (Villiers 1974) and repair the membrane. In contrast to our results, Bonner (1991) in southern pines, Singh et al. (1996) in *Gleditisia trichanthos* and *Robinia pseudacacia* and Tammela et al. (2000) in *Pinus sylvestris* reported higher EC and lower germination percentages for stored seeds. Varghese and Naithani (1997) also reported that the EC of leachate showed a remarkably gradual increase with deterioration due to age of neem seeds.

WSS and amino acids were measured from leachate obtained from seeds of all mother tree age classes (Table 1). The maximum WSS and amino acids were released when seeds were fresh. Both declined significantly as storage duration increased. Germination percentage also declined with duration of storage for seeds of all mother tree age classes. These results are in accordance with the findings of Agrawal (1977) for paddy seeds. He reported that the amount of WSS in seed leachate declined with seed deterioration. Mccomb and Winstead (1964) reported that reduced levels of several amino acids in seeds were due to fungal invasion.

A gradual decline was recorded in the phospholipid content of seed oil as storage duration increased. Fresh seeds showed higher activity than stored seeds (Table 2). These results are in line of earlier reports for many species, *viz*. Stewart and Bewlay (1980) and Priestley and Leopold (1979) for *Glycine max* (Soyabean), Pearce and Abdel Samad (1980) in *Arachis hypogiea* (Pea nut) and Halder et al. (1983) in *Helianthus annuus* (Sunflower). They related declines in phoshpholipid content with reduced seed viability. Similar results were reported by Pukacka (1998) for *Acer saccharinum* (Silver maple), Pukacka and Czubak (1998) for *Acer pseudoplatanus*, and Tammela et al. (2000) for *Pinus sylvestris* seeds.

Table 1. Effect of storage on cell membrane permeability (leachate) of different mother tree age classes of neem seed. Values are means of four replications. Seeds (1g) were soaked into 50 mL double distilled water incubated at 25±1°C for 24 h.

Age classes	Electrical conductivity (μS/g)			Water soluble sugar (mg/g)			Amino acids (mg/g)		
	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days
I	267.56[a]	153.37[b]	147.82[b]	19.48[a]	3.13[b]	2.06[c]	5.40[a]	4.46[b]	4.03[d]
II	279.26[a]	188.82[b]	144.02[b]	19.54[a]	3.56[b]	2.40[c]	5.36[a]	4.29[c]	4.17[c]
III	268.91[a]	171.38[b]	149.75[b]	19.85[a]	3.33[b]	2.16[c]	5.36[a]	4.49[b]	4.27[c]
IV	295.01[a]	195.17[b]	161.56[b]	19.96[a]	4.17[b]	2.29[c]	5.35[a]	4.41[b]	3.98[d]

Values followed by different letters are significantly different (p <0.05)

Table 2. Effect of seed storage on cell membrane integrity {phospholipid and anti-oxidants (α-tocopherol) in oil and lipid peroxidation (in embryo)} of neem seeds of different mother tree age classes. Values are means of four replications.

Age classes	Phospholipid (OD at 710 nm)			α-Tocopherol (OD at 520 nm)			Lipid peroxidation {OD at (600–535 nm)}		
	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days
I	0.073[a]	0.036[b]	0.032[c]	0.0100[a]	0.0020[c]	0.00	0.0076[d]	0.0200[b]	0.0268[a]
II	0.080[a]	0.033[b]	0.030[c]	0.0105[a]	0.0020[c]	0.00	0.0066[d]	0.0191[b]	0.0255[a]
III	0.086[a]	0.038[b]	0.034[c]	0.0105[a]	0.0020[c]	0.00	0.0073[d]	0.0213[b]	0.0248[a]
IV	0.093[a]	0.042[b]	0.034[c]	0.0080[b]	0.0010[c]	0.00	0.0087[c]	0.0220[b]	0.0253[a]

Values followed by different letters are significantly different (p <0.05)

Lipid peroxidation as manifested by changes in fatty acid saturation or malondialdehyde (MDA) production has been assumed to be the primary mechanism by which the putative free radical injury is imposed on plant membranes. But another mode of damage could be brought by nucleophilic attack by the superoxide radical (Niehaus 1978). Free radical damage to cellular membrane could proceed by de-esterification of membrane phospholipids and the resultant accumulation of fatty acids would lead to reduced membrane function. Table 2 also shows absorbance of lipid peroxidation in seeds of various mother tree age classes. These results are in accordance with the findings of Varghese and Naithani (2002). They reported the levels of lipid peroxidation products were higher in axes compared to cotyledons of 100% viable seeds.

Table 2 shows absorbance of antioxidant (α-tocopherol) content in oil obtained from seeds of all mother tree age classes. Fresh seeds had higher concentrations of antioxidants while dead seeds showed minimal activity of antioxidants. Similar results were reported by Tammela et al. (2000) for *Pinus sylvestris* in aged seeds and by Pukacka and Ratajczak (2007) for *Fagus sylvatica* during storage. They tested 1, 18 and 24 year-old seeds and recorded declines in antioxidant (α- tocopheral) content with increasing age of seeds. Loss of α-tocopherol content was also reported due to age of wheat, sesame, and cotton seeds although in soybeans the results have been more or less contradictory (Priestley et al. 1980; Wilson et al. 1986).

Protein is the main form of the nitrogen storage in food reserves in seeds. During storage, proteins become less soluble and are degraded into free amino acids (Anderson 1946). Several investigations have shown that reductions in vigor and viability were closely associated with declines in protein synthesis (Roberts et al. 1973; Ghose and Chaudhuri 1984). The protein content of neem seed cake is 25.4% (Anon 1978) while Parmar and Katkar (1996) reported 17.03% to 36% crude protein and 26% carbohydrate in neem cake. We documented a gradual decline in total protein content with increasing seed age (Table 3). Total protein content gradually declined with increased storage duration in seeds of all the mother tree age classes. Similar results were reported by Suszka (1975). He reported reductions in protein synthesis and oxygen uptake that paralleled declines in germination of stored seeds. Declines in protein content and viability with increasing storage duration were reported by Mani (1997) for *Acacia nilotica* and *Acacia leucophloea*, Nautiyal and Prohit (1985) for *Shorea robusta*, Umarani (1999) for *Casuarina equisetifolia*, and Vijayaragavan and Vanangamudi (2003) for *Albizia lebbeck*. Many authors have documented reduction of protein due to infestation during storage. Cherry et al. (1974) reported that the decrease in protein content was due to the hydrolysis of protein into its simple forms by the activities of hydrolytic enzymes produced by fungi. Tripathi et al. (1996) reported sharp declines in protein content in infested neem seeds.

There was a gradual decline in total sugar content with increasing storage duration in seeds of all mother tree age classes (Table 3). Total sugar content was higher in fresh seeds and declined

significantly in seeds of all mother tree age classes during the storage period. These results are in accordance those of Nema (1983), Prasad (1983) and Balkrishnan and Nair (1983). A similar trend was observed in total starch of seeds of all mother tree age classes during storage (Table 3). Total starch was highest in fresh seeds of all mother tree age classes. All mother tree age classes showed significantly reduced total starch content during storage. These results corroborate the findings of Singh et al. (1996) for *Jatropha curcas* and Tripathi et al. (1996) for neem seeds. They reported sharp reductions in starch content in infested seeds.

Declines in germination and mean germination time (MGT) during storage are listed in Table 4. All seeds lost their viability after 65 days of storage.

Age class II showed maximum germination percentage (96.25%) followed by age class III (93.5%) and age class I (92.25%) (Table 4). Significantly lower germination percentage was observed for seeds of age class IV trees. Significant reduction in germination percentage was observed in seeds of all age classes after 35 days of storage. Significantly lower germination was observed in age class II (37.75%) followed by age classes I, III and IV with 31.5%, 15.25% and 5.0%, respectively after 35 days of storage (Table 4). Age class IV showed higher MGT (poor) as compared to age classes I, II and III in fresh seeds. Age class II showed slow increase in MGT (18.51 days) followed by age classes I, III and IV with 19.72, 20.86, and 21.61 days respectively after 35 days of storage. However, age class II showed minimum MGT followed by age I and III as compared to age class IV after 35 days of storage (Table 4). Age class IV exhibited significantly fastest increase in MGT after 35 days of storage. The GV of fresh seeds was significantly higher in age class II, followed by age classes I and III. Age class IV showed lower GV. GV declined significantly with increasing storage duration (Table 4). Seeds of all age classes of trees showed good germination (except for age class IV) when their oil showed lower acid value, sap value and ester value. No seeds germinated after 65 days of storage. Germination was higher for fresh seeds and reduced as biochemicals in the seeds deteriorated with increasing storage duration. Patterns of seed deterioration were similar across all seed lots.

Table 3. Effect of storage on food reserves (total protein, sugar and starch) of neem seeds of different mother tree age classes. Values are means of four replications.

Age classes	Total protein (mg/g)			Total sugar (mg/g)			Total starch (mg/g)		
	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days
I	174.83 [a]	117.81 [c]	85.63 [d]	25.17 [a]	7.48 [b]	1.07 [c]	20.59[a]	6.73[b]	0.96[c]
II	186.25 [a]	124.22 [c]	89.85 [d]	25.36 [a]	7.15 [b]	0.78[c]	22.83[a]	6.44[b]	0.70 [c]
III	179.56 [a]	120.47 [c]	94.30 [d]	25.20 [a]	7.01 [b]	0.78[c]	22.68[a]	6.31[b]	0.70 [c]
IV	159.53 [b]	113.44 [c]	64.22 [e]	26.79 [a]	6.05 [b]	0.55[c]	24.11 [a]	5.45[b]	0.49 [c]

Values followed by different letters are significantly different (p<0.05)

Table 4. Effect of storage on germination of neem seeds obtained from various different mother tree age classes. Values are means of four replications.

Age classes	Germination (%)			Mean germination time (MGT, days)			Germination value (GV)		
	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days	Fresh/ 0 days	35 days	65 days
I	92.25 [b] (73.9)	31.5 [e] (34.2)	0	11.63 [a]	19.72 [d]	22.00 [g]	37.79 [b]	2.44 [e]	0
II	96.25 [a] (79.0)	37.75 [d] (37.9)	0	11.47 [a]	18.51 [c]	22.00 [g]	39.88 [a]	4.81 [d]	0
III	93.5 [b] (75.3)	15.25 [f] (23.0)	0	11.68 [a]	20.86 [e]	22.00 [g]	38.18 [b]	0.60 [f]	0
IV	74.75 [c] (59.9)	5.00 [g] (12.9)	0	13.96 [b]	21.61 [f]	22.00 [g]	23.50 [c]	0.07 [f]	0

Values followed by different letters are significantly different (p <0.05)

Conclusion

Neem seed lost its viability and vigor after 35 days when stored at room temperature. No germination was observed in seeds obtained from any mother tree age class after 65 days of storage. Phospholipid and α-tocopherol declined with increasing storage time, and lipid peroxidation in terms of malondialdehyde (MDA) increased with increasing storage duration. These trends led to development of rancidity of oil in the seed, which led to cell membrane disfunction. Cell membrane permeability tests for electrical conductivity (EC), water soluble sugars (WSS) and amino acids (AA) all showed declines with increasing duration of storage. Reduction of cell membrane permeability and released amounts of solute may be due to repair during imbibition of seeds. Reduction of food (protein, total sugar and total starch) was recorded with increasing duration of the storage period. Thus we conclude that deterioration in seeds obtained from all the mother tree age classes during storage, the loss of viability and vigor of seed are strongly related to loss of protein, sugars, starch, indicating weakening of cell integrity in terms of phospholipids, α-tocopherol and lipid peroxidation.

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References

Abdul-Baki AA, Anderson JD. 1970. Viability and leaching of sugars from germinating barley. *Crop Science*, **10**: 31–34.

Abdul-Baki AA, Anderson JD. 1972. Physiological and biochemical deterioration of seeds. *In*: Kozlowski T.T. (ed.), *Seed biology,* Vol. 2. New York: Academic Press, pp. 283–315

Agrawal PK, Dadlani M. 1992. Membrane permeability: A measure of seed quality. In: Agrawal P.K. and Dadlani M. (eds.), *Techniques in seed science and technology*. New Delhi: South Asian Publisher, International Book Company Absecon Highlands, N.J. pp. 127–133

Agrawal PK. 1977. Germination, fat acidity and leaching of sugars from five cultivars of paddy rice (*Oryza sativa*) seeds during storage. *Seed Science and Technology*, **5**: 489–498.

Anderson JD. 1946. *Enzymes and their role in wheat technology*. New York: Inter science Publishers Inc., p.371.

Anon. 1978. Progress report of ICFRE Scheme. All India coordinated research project for investigation on agricultural byproducts and industrial waste materials for evolving economic rations for livestock. Jawahar Lal Nehru Krishi Vishva Vidyapith, Jabalpur, India.

AOAC (Official Methods for Analysis). 1990. Association of official analytical chemists. Incorporation Virginia, USA, Vol. 2. 15th Ed., pp.951–986.

Balkrishnan B, Nair MC. 1983. Observation on the post infectional changes of rice plants infected by the sheath rot fungus, *Acrocylindrium oryzae* saw. *Agriculture Research Journal of Kerala*, **21**: 73–74.

Barber AA, Bernheim F. 1967. Lipid peroxidation: its measurement, occurrence, and significance in animal tissues. In: B.L. Strehler (ed.), *Advanced Genetic Research*, Vol. **2.** New York: Academy Press, pp. 355–403.

Bewley JD, Black M. 1982. *Physiology and Biochemistry of seeds (Vol. 2)*. New York: Springer-Verlag, pp.1–58.

Bewley JD. 1986. Membrane changes in seeds as related to germination and the perturbations resulting from deterioration in storage. *In*: (M.B. McDonald and C.J. Nelson (eds.), *Physiology of seed deterioration*. Madison, WI: Crop Science Society of America, pp.27–46.

Bonner FT. 1991. Estimating seed quality of southern Pines by leachate conductivity. Research Paper SO-263, New Orleans, LA, USDA Forest Service, Southern Forest Experiment Station. pp.1–4.

Bradnock WR, Matthews S. 1970. Assessing field emergence potential of wrinkle seeds peas. *Horticulture Research*, **10**: 50–58.

Cherry JP, Mayre RV, Ory RL. 1974. Protein content and enzymes in rare seeds of *Arachis hypogae* L. *Physiological Plant Pathology*, **41**: 425–439.

Ching TM, Scoolcraft I. 1968. Physiological and chemical differences in aged seeds. *Crop Science*, **8**: 407–409.

Colowic SP, Kaplan WC. 1957. *Method in Enzymology (Vol. III)*. New York: Academic Press, p.468.

Dadlani M, Agrawal PK. 1983. Mechanism of soybean seed deterioration. *Plant Physiology and Biochemistry*, **10**: 23–30.

Djavanshir K, Pourbeik H. 1976. Germination value –a new formula. *Silvae Genetica*, **25**: 79–83.

Dubois M, Gilles KA, Hamilton JK, Robers PA, Smith F. 1956. Colorimetric method for determination of sugar and related substrates. *Annals of Chemistry*, **28**: 350–356.

Ezumah BS. 1986. Germination of storage of neem (*Azadirachta indica* A. Juss.). *Seed Science and Technology*, **14**: 593–600.

Fick GL, Hibbard RP. 1952. A method for determining seed viability by electrical conductivity measurements. *Michigan Academy of Science arts and Letters*, **5**: 95–103.

Fielding JL, Goldworthy A. 1980. Tocopherol levels and ageing in wheat grains. *Annals of Botany*, **46**: 453–456.

Ghose B, Chaudhari MM. 1984. Ribonucleic and breakdown and loss of protein synthetic capacity with loss of viability of rice embryo. *Seed Science and Technology*, **12**: 669–677.

Halder S, Kole S, Gupta K. 1983. On the mechanism of sunflower seed deterioration under two different type of accelerated ageing. *Seed Science and Technology,* **11**: 331–339.

Helmer JD, Delouche JC, Leinhard M. 1962. Some indices of vigor and deterioration in seeds of crimson clover. *Proceeding Association Official Seed Analysis*, **52**: 154–160.

Heydecker W. 1972. Vigor. *In*: E.H. Roberts (ed.), *Viability of seeds*. Syracuse, N.Y.: Syracuse University Press, pp. 209–252

Hsu FH, Lin JB, Chang SR. 2000. Effects of water logging on seed germination, electric conductivity of seed leakage and developments of hypocotyls and radicle in sudangrass. *Botanical Bulletin of Academia Sinica*, **41**: 267–273.

Kaloyereas SA.1958. Rancidity as a factor in the loss of viability of pine and other seeds. *Journal of the American Oil Chemists Society*, **35**: 176–179.

Koostra PT, Harrington JF. 1969. Biochemical effects of age on membrane lipids of *Cucumis sativus* L. seed. *Proceedings of the International Seed Testing Association,* **34**: 329–340.

Lowery OH, Rosebroagh NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265–275.

Maccomb CL, Winstead NN. 1964. Changes in sugar and aminoacids of cucumber fruits infected with *Pythium aphanidermatum*. *Phytopathology*, **54**: 233–234.

Mani G. 1997. Seed Technology studies in *Acacia nilotica* (Linn.) and *Acacia leucocephloea* (Roxb.) M. Sc. (Ag.) Thesis, Department of Seed Technology, Tamil Nadu Agricultural University, Coimbatore-641003.

Matthews S, Bradnock WT. 1967. The detection of seed samples of wrinkled-seeded peas (*Pisum sativum* L.) of potentially low planting value. *Proceedings of the International Seed Testing Association*, **32**: 555–563.

Matthews S, Whitbread R. 1967. Factors affecting pre-emergence mortality in peas. I. An association between seed exudates and the incidence of pre-emergence mortality in wrinkle-seeded peas. *Plant Pathology*, **17**: 11–17.

Mukhtar ND, Laidman DL. 1982. Mineral ion transport in the embryos of germinating wheat (*Triticum aestivum*). *Journal of Experimental Botany*, **33**: 643–655.

Nagaveni HC, Ananthapadmanabha HS, Rai SN. 1987. A note on the extension of viability of *Azadirachta indica* A. Juss. *Myforest,* **23**: 245.

Nautiyal AR, Purohit AN. 1985. Seed viability in Sal I. Physiological and biochemical aspects of seed development in *Shorea robusta*. *Seed Science and Technology*, **13**: 59–68.

Nema DK. 1983. Changes in sugar of apple due to *Alternaria alternata* infection. *Indian Phytopath*, **36**: 624–629.

Niehaus WJJr. 1978. A proposed role of superoxide anion as a biological nucleophile in the desertification of phospholipid. *Bioorganic Chemistry*, **7**: 77–84.

Parmar BS, Katkar CM. 1996. Commercialization. *In*: N.S. Randhawa and

B.S. Parmar (eds.), Neem-1993, Publisher, Society of Pesticides Science India and New Age International (P) Ltd. pp.318–332

Pearce RS, Abdel Samad IM. 1980. Change in fatty acid content of polar lipids during ageing of seeds of peanut (*Arachis hypogiea* L.). *Journal of Experimental Botany*, **31**: 1283–1290.

Pearson D. 1970. *The Chemical Analysis of Foods*. New York: Chemical Publishing Co., p.535.

Pehap A. 1973. Selected papers in gerontology of some forest seeds. Research Notes, Skosgshogskolan. Royal College of Forestry, Stockholm. 1–175 pp.

Perry CA. 1987. *Handbook of Vigor Test Methods. 2nd ed*. Zurich: ISTA.

Pesis E, TIMOTHY JN. 1983. Viability, vigor and electrolytic leakage of muskmelon seeds subjected to accelerated ageing. *Horticulture Science*, **18**: 242–244.

Powell AA, Mathews S. 1977. Deteriorative changes in pea seeds (*Pisum sativum* L.) stored in humid or dry conditions. *Journal of Experimental Botany*, **28**: 225–234.

Powell AD, Dulson J, Bewley JD. 1984. Changes in germination and reparatory potential of embryos of Grand Rapid lettuce seeds during long term imbibed storage, and related changes in the endosperm. *Planta*, **162**: 40–45.

Powell AD, Leung DWM, Bewley JD. 1983. Long term storage of dormant Grand Papids lettuce seeds in the imbibed state: physiological and metabolic changes. *Planta*, **159**: 182–188.

Prasad BK. 1983. Free sugar and amino acids contents of coriander infected with stem gall disease. *Indian Phytopathology*, **36**: 150–151. .

Presley DA, Mcbride MB, Leopold AC. 1980. Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. *Plant Physiology*, **66**: 715–719.

Priestley DA, Leopold AC. 1979. Absence of lipid oxidation during accelerated aging of soybean seeds. *Plant Physiology*, **63**: 726–729.

Priestley DA. 1986. *Seed ageing: Implication of seed storage and persistence in the soil*. Ithaca: Cornell University Press, p. 304.

Pukacka S, Czubak A. 1998. The effect of desiccation on viability and membrane lipid composition of *Acer pseudoplatanus* seeds. *Acta Societatis Botanicorum Poloniae*, **67**: 249–252.

Pukacka S, Ratajczak E. 2007. Age related biochemical changes during storage of beech (*Fagus sylvatica* L.) seeds. *Seeds Science Research*, **17**: 45–53.

Pukacka S. 1998. Changes in membrane fatty acids composition during desiccation of seeds of *Acer saccharinum* (silver maple). *Seed Science and Technology*, **26**: 535–540.

Raheja RS, Kaur C, Singh A, Bhatia IS. 1973. New colorimetric method for the quantitative estimation of phospholipid without acid digestion. *Journal Lipid Research*, **14**: 695–697.

Rawat MMS, Thapliyal RC. 2003. Endogenous rhythm in seed germination of *Dendrocalamus strictus*. *Seed Science and Technology*, **31**: 21–27.

Roberts BE, Payne PI, Osborne DJ. 1973. Loss of activity of protein synthesizing systems in vitro associated with a loss of viability. *Biochemistry Journal*, **131**: 275–286.

Simon EW, Raja Harun RM. 1972. Leakage during seed imbibition. *Journal of Experimental Botany*, **23**: 1076–1085.

Singh P, Singh O, Chandra V. 1996. Estimating seed quality in hard seeded leguminous trees by accelerated aging and leachate conductivity. *Indian Forester*, **122**: 415–418.

Stewart RRC, Bewlay JD. 1980. Lipid peroxidation associated aging of soybean axes. *Plant Physiology*, **65**: 245–248.

Suszka B. 1975. Cold storage of already after ripened beech (*F. silvatica*) seeds. *Arboretum Kornickie*, **20**: 299–315.

Takayanagi K, Murakami K. 1968. Rapid germinability test with exudates from seeds. *Nature*, **218**: 493–494.

Tammela P, Hopia A, Hiltunen R, Vuorela H, Nygren M. 2000. Ageing in *Pinus sylvestris* L. seeds: Changes in viability and lipids. *Biochemistry Society Trans*, **28**: 878–879.

Tappel AL. 1965. Free radical peroxidation damage and its inhibition by Vitamin E and selenium. *Fed Proc Fed Am Socs Exp Biol*, **24**: 73–78.

Tripathi YC, Gupta PK, Rathore M. 1996. Biodeterioratve effect on chemical composition of neem seeds. *Bulletin of Pure and Applied Science*, **15B**: 121–124.

Umarani R. 1999. Studies on the physiological and biochemical basis of seed germination and deterioration in *Casuarina equisetifilia*, Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore-641003.

Verghees B, Naithani SC. 1997. Physiological and biochemical changes associated with ageing in neem seed. In: *IUFRO Symposium on innovation in forest tree seed science and nursery technology*, Abstract Book organized by School of life science, Pt. Ravishankar Shukla University, Raipur-492010, India. pp.157–158

Varghese B, Naithani SC. 2002. Desiccation induced changes in lipid peroxidation, superoxide level and antioxidant enzymes activity in neem (*Azadirachta indica* A. Juss.) seeds. *Acta Physiol Plant,* **4**(1): 79–87.

Vijayaragavan A, Vanangamudi K. 2003. Effect of seed source and period of storage on germination and biochemical activity of *Albizia lebbeck* (L) Benth. Seeds. *My Forest*, **39**: 341–351.

Villiers TA. 1974. Seed aging: Chromosome stability and extended viability of seeds stored fully imbibed. *Plant Physiology*, **53**: 875–878.

Walker BL, Singer SJ. 1975. Effect of processing on the tocopherol content of rapeseed oil. *Canadian Institute of Food Science and Technology Journal,* **8**: 179–180.

Wilson DO, Mcdonald JrMB. 1986. The lipid peroxidation model of seed ageing. *Seed Science and Technology*, **14**: 269–300.

Yaklich RW, Abdul-Baki AA. 1975. Variability in metabolism of individual axes of soybean seeds and its relationship to vigor. *Crop Science*, **15**: 424–426.

Yamauchi R, Matsushita S. 1976. Quantitative changes in tocopherols and their intracellular distribution in cotyledons during soybean germination. *Nippon Nogeikagakn Kaishi,* **50**: 525–528.